RTase III Primer Flexible All-in-One Mix (with dsDNase)

REF: EG24102S

Storage Condition

-20℃

Components

Component	Amount
RTase III Primer Flexible All-in-One Mix	400 μl
Oligo(dT)$_{20}$VN (50 μM)	100 μl
Random hexamers (50 μM)	100 μl
dsDNase	2×50 μl
10× dsDNase Buffer	200 μl
Nuclease-Free Water	2×1 ml

Description

RTase III Primer Flexible All-in-One Mix is a high-quality, efficient, and convenient one-step cDNA synthesis premix. It is designed to minimize contamination and contains all the necessary components for first-strand cDNA synthesis, including M-MLV GIII Reverse Transcriptase and its reaction buffer, RNase inhibitor, dNTPs, and random primers-all the necessary components. Additionally, it requires the addition of RNA templates, primers, and water to initiate the reaction. This kit can be used with different types of reverse transcription primers to meet diverse experimental needs. Depending on the experimental design, Oligo(dT)$_{20}$VN, Random hexamers, or Gene Specific Primers can be selected. This reverse transcription premix allows for the generation of cDNA up to 12 kb in size within 15 minutes.

RNA extracted from cells often contains genomic DNA contamination. If the genomic DNA is not removed before reverse transcription, both the genomic DNA and cDNA will be amplified during downstream qPCR reactions (especially when the primers are designed on the same exon), thus affecting the accuracy of gene expression quantification. This kit utilizes dsDNase to efficiently remove genomic DNA contamination. dsDNase can specifically digest double-stranded DNA (dsDNA or the DNA strand in DNA-RNA hybrid chains) and is thermally sensitive, rapidly and irreversibly inactivated at the reverse transcription temperature. Compared to the traditional method of using DNase I to remove genomic DNA contamination, dsDNase does not require the addition of EDTA for inactivation, saving experimental time and reducing inhibition of the reverse transcription reaction.

Protocol

1. Total RNA

(1) Genomic DNA removal

① Prepare the following reaction mixture on ice:

Reagent	Amount
Total RNA[a]	50 ng~1 μg
dsDNase	1 μl
10× dsDNase Buffer	1 μl
Nuclease-Free Water	To 10 μl

a. It is recommended to use RNA extracted from a kit as a template.

② Mix gently and spin down.

③ Incubate at 37℃ for 2 minutes to remove genomic DNA contamination.

Note: If the RNA has severe contamination of genomic DNA, the incubation time at 37℃ can be appropriately extended to 5 minutes.

④ Incubate at 65°C for 2 minutes to inactivate dsDNase, and then place on ice.

(2) First-strand cDNA synthesis

① Prepare the following reaction mixture on ice:

Reagent	Amount（Experimental group）
The reaction product of "Experiment (1)"	10 μl
RTase III Primer Flexible All-in-One Mix	4 μl
Oligo(dT)$_{20}$VN (50 μM)	1 μl
Or Random hexamers (50 μM)	1 μl
Or Gene Specific Primers (50 μM)	0.1 μl
Nuclease-Free Water	To 20 μl

② Mix gently and spin down.

③ Incubate at 55℃ for 15 minutes.

Note: If the template RNA does not contain a poly(A) tail, you may pre-incubate at 25℃ for 10 minutes.

④ After the reaction is complete, incubate at 85 ℃ for 5 minutes to terminate the reaction.

⑤ Quickly place the obtained cDNA on ice for subsequent experiments.

Note:

1. For the follow-up cloning experiment, if the RNA comes from eukaryotes, only Oligo (dT)$_{20}$VN is needed. Adding Random hexamers will reduce the yield of full-length cDNA. If the RNA comes from prokaryotes, only Random hexamers or Gene Specific Primers are needed.

2. For the follow-up qPCR experiment, please add both Oligo(dT)$_{20}$VN and Random hexamers to obtain cDNA with uniform reverse transcription efficiency at different positions of mRNA.

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2. miRNA

(1) Genomic DNA removal

① Prepare the following reaction mixture on ice:

Reagent	Amount
miRNA	10 pg~200 ng
dsDNase	1 μl
10× dsDNase Buffer	1 μl
Nuclease-Free Water	To 10 μl

② Mix gently and spin down.

③ Incubate at 37℃ for 2 minutes to remove genomic DNA contamination.

Note: If the RNA has severe contamination of genomic DNA, the incubation time at 37°C can be appropriately extended to 5 minutes.

④ Incubate at 65°C for 2 minutes to inactivate dsDNase, and then place on ice.

(2) First-strand cDNA synthesis

① Prepare the following reaction mixture on ice:

Reagent	Amount（Experimental group）
The reaction product of "Experiment (1)"	10 μl
RTase III Primer Flexible All-in-One Mix	4 μl
Stem-loop primer(5 μM)[b]	1 μl
Nuclease-Free Water	To 20 μl

b. The recommended final concentration of the stem-loop primer is 0.25 μM, which can be adjusted within the range of 0.1 to 0.5 μM.

② Mix gently and spin down.

③ Incubate at 55℃ for 15 minutes.

④ After the reaction is complete, incubate at 85℃ for 5 minutes to terminate the reaction.

⑤ Quickly place the obtained cDNA on ice for subsequent experiments.

miRNA stem-loop primer and qPCR primer design

1. Stem-loop RT Primer Design: Based on a universal stem-loop structure, only the last 6 bases need to be modified according to different miRNA sequences. The universal stem-loop structure sequence is: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC.

2. Taking miR-1-5p as an example, its sequence is CAUACUUCCUUACAUGCCCAUA. To design the stem-loop RT primer, simply add the reverse complementary sequence of the last 6 bases of the miRNA 3' end to the universal stem-loop sequence, which would be GTCGTATCCAGTGCAGGGTC CGAGGTATTCGCACTGGATACGAC"TATGGG".

3. qPCR Upstream Primer Design: The remaining portion of the miRNA sequence after removing the last 6 bases from the 3' end serves as the upstream primer (replace U with T). For example, the upstream primer for miR-1-5p is: CATACTTCCTTACATG. Check the melting temperature (Tm) of the primer. If the Tm is low, add GC at the 5' end to bring the Tm value close to 60°C . Therefore, the upstream primer for miR-1-5p can be designed as GCCGCCATACTTCCTTACATG, with a Tm of 60.3°C .

4. The downstream primer is universal, and the sequence is GTGCAGGGTCCGAGGT.

5. After designing the primers, it is necessary to test their specificity through preliminary experiments. Typically, a melting curve analysis is performed to detect the specificity of the primers. Additionally, it is best to perform electrophoresis on the PCR products to check whether the product is shown as a single band (since the product length is short, agarose gel with a concentration of 3% or higher is required).

Notice

1. Before using any reagents, please gently invert them upside down to mix evenly, try to avoid creating bubbles, and centrifuge briefly before use.
2. To prevent RNase contamination, please keep the experimental area clean.
3. Wear clean gloves and masks during operation.
4. Ensure that all consumables such as centrifuge tubes and pipette tips are RNase-free.

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